MULTIPLE PATERNITY ANALYSIS IN A HYBRID ZONE

An Undergraduate Research Scholars Thesis

by

JANAE ALYSE SAMANTHA RAPP

Submitted to Honor and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Gil Rosenthal

May 2014

Major: Biology

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ABSTRACT

Multiple Paternity Analysis in a Hybrid Zone. (May 2014)

Janae Alyse Samantha Rapp
Department of Biology
Texas A&M University

Research Advisor: Dr. Gil Rosenthal
Department of Biology

Hybridization is an important evolutionary process. Hybrid zones can illustrate mechanisms which lead to introgression. Two live bearing freshwater fish, *Xiphophorus malinche* and *X. birchmanni* have formed several natural hybrid zones. The females of both species mate several times with several males (polyandry). Polyandry increases genetic diversity and the opportunity for sexual selection. To analyze levels of multiple paternity, we will genotype parents and offspring from one population using microsatellite markers, allowing for easy paternity assignment. Using the program GERUD, we will determine the number of sires for each female's offspring. We expect, like parental species, that hybrid females will display a high level of multiple paternity. By studying multiple paternity in this hybrid zone; consequences of polyandry, such as increased opportunities for sperm competition or other mechanisms promoting post copulatory sexual selection, can be studied in this system in the future.

CHAPTER I

INTRODUCTION

Hybridization in animals is often thought of as nothing more than an accident or mistake—individuals mating with the wrong mate and suffering a fitness cost as a result. However, the collision of genomes can introduce new genetic and phenotypic variation into a population. Depending on the environment, this may lead to increased individual fitness and allow for integration of novel gene combinations via gene flow between divergent species(*1*,*2*). More work is necessary in order to understand how hybridization occurs and how mechanisms of selection work to promote hybridization or the maintenance of distinct species (5). The hybrid zones between sister species *X. malinche and X. birchmanni* in east central Mexico serve as a powerful natural model of dynamic interactions in hybridization and speciation. Gil Rosenthal's lab at Texas A&M University has focused primarily on *pre-mating sexual selection* via mate choice, leading to an understanding of the communication signals important to the reproductive success of these fish and of the role communication plays in hybridization(*4*). We also know that adult *hybrids of these two species are viable* and generally show no sign of reduced fitness(*5*). *As of yet, no work has been done to investigate post-mating/pre-zygotic (fertilization) barriers in this system.* This is in spite of the fact that this system has potential as an excellent vertebrate model for post-copulatory sexual selection: *Xiphophorus* are live-bearing fish that, like other poeciliids, may store sperm from multiple males within their reproductive tract for up to 10 months, meaning that sperm competition and/or postcopulatory female choice probably occur(*6*).

The first step in assessing the presence and possible importance of post-copulatory sexual selection in areas of hybridization is determining levels of multiple paternity in the hybrid zones. Previous work in *X. birchmanni* revealed that these fish have very high levels of multiple paternity (Paczolt et al, in prep), and one hypothesis is that within a hybrid zone, the more permissive hybrid females will also have a high level of multiple paternity in their broods. However, it may be that they actually display a lower level of multiple paternity; variance in sperm/egg interactions between parentals and hybrids and/or other post-copulatory mechanisms may mean that, although a female mates multiply, not all of these matings will be successful. To begin to have some idea of what may be happening, an analysis of paternity in hybrid populations is necessary. Work informed by this study will help in the understanding of the complex interactions that occur within hybrid zones; interactions that determine the evolutionary fate of both the hybrids and the parental species involved.

CHAPTER II

METHODS

Gravid females were collected from a hybrid zone displaying an assortative mating structure (distinct groups of hybrids and parentals in the population) in June of 2012 during the dry season. All gravid females from one pool in the Rio Calnali were collected and sacrificed in tricane mesylate, as per standard lab procedure (5). Offspring were dissected out in Fall of 2012 in N=35 females, with an average of N=27 offspring per female. DNA extraction techniques will be performed using a DNeasy tissue kit (Qiagen Inc.). Once the DNA has been extracted, PCR will be performed to amplify a series of six informative microsatellite markers (5). Samples will then be sent off to the University of Maryland for fragment analysis, and results will be genotyped using Peakscanner (5). I will then analyze this data using GERUD 2.0 (7).

CHAPTER III

RESULTS

I looked for a concentration of DNA greater than or equal to 40 ng/µL with the absorbance ratio of 260/280 nm being 1.8 or greater. (1.8 is accepted to be pure for DNA). After extracting the DNA for the entire population of hybrid offspring and females, the average DNA concentration across all samples was 592.83 ng/µL with a standard deviation of 276.9 ng/µL. With the determined concentration of DNA per sample, they were then diluted to have a desired concentration of 40 ng/µL and a desired volume of 100 µL. The sample volume was determined using the formula (Desired Concentration 40 ng/ µL * Desired Volume 100 µL)/ the actual concentration of the sample. The TE volume was calculated by subtracting the sample volume from 100. The microsatellite marker Msd072 showed successful amplification as seen by the dark bands at 130-270 bp range (Paczolt et al. in review). The optimized protocol for this primer was determined to be as follows: 8.336µl of distilled water, 1.5µl PCR buffer, 1.5µl of 2µM dNTP, 1.2µl 25mM $MgCl_2$, 0.22µl of Msd 072 Forward primer, 0.22µl of Msd 072 Reverse primer, and 0.144µl of TAQ polymerase was added into each well of a 96 well plate. The program used on the thermal cycler were as followed for Msd 072; step one was 94ºF for 2:00mins, step two was 94ºF for 0:15secs, step three 60ºF for 0:30secs, step four 72ºF 1:00min, step five went back to step two through five and repeated 35 times, and step six was 72ºF for 4:00 minutes.

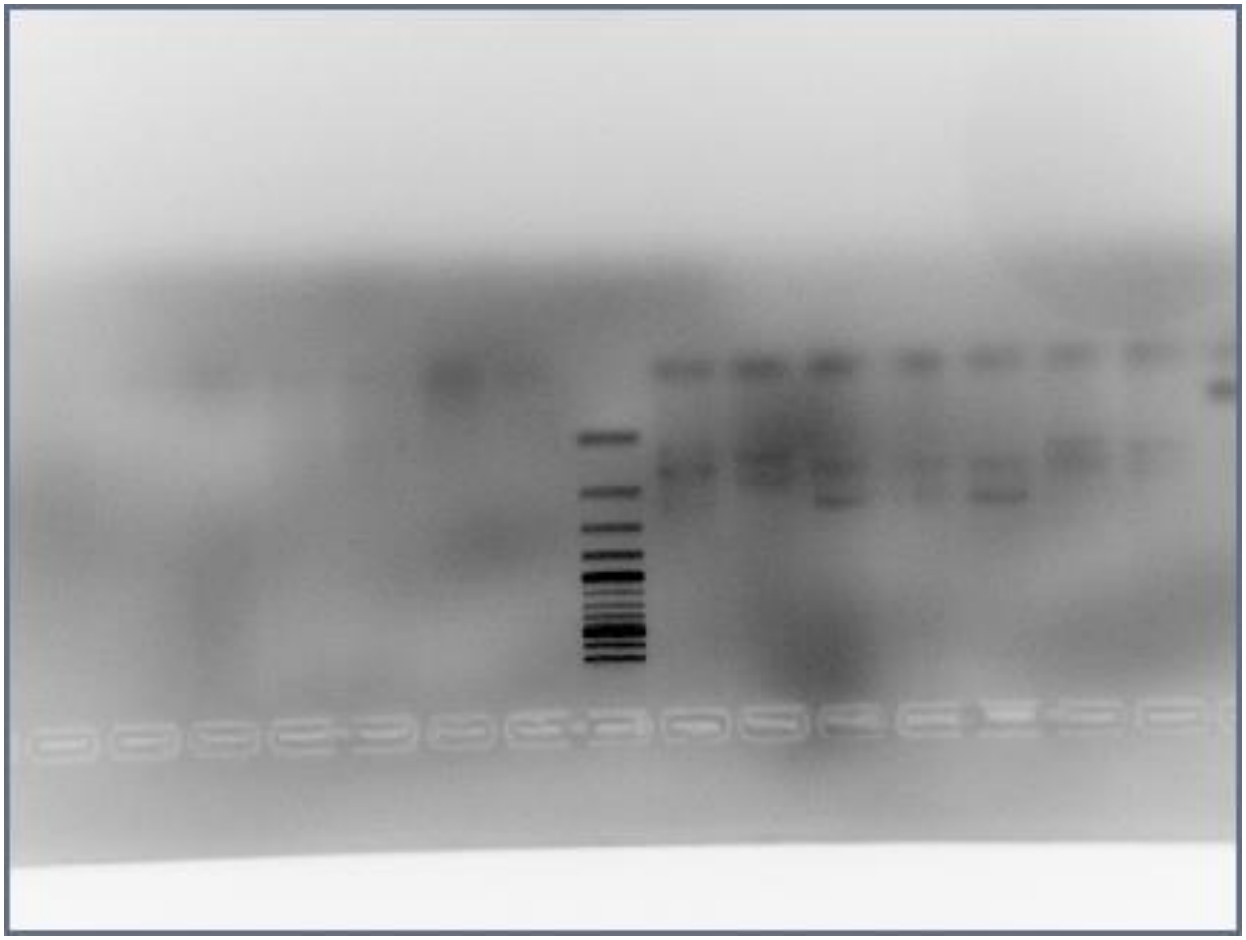

Figure 1: The figure at the right is a gel image for a test run of MSD072. Samples from left to right are as follows: New England Biolabs 100 bp ladder, 2 *X. birchmanni* from COAC site, 3 *X. malinche* from CHIC site, 2 hybrid females from the study population of SYCA, and a negative control. Localities of sites are found in Culumber et al. 2011.

CHAPTER IV

CONCLUSION

DNA Extraction

The average concentration of DNA for the entire population was 592.83 ng/µL with a standard deviation of 276.9 ng/µL. This indicated that a large amount of DNA was present in the samples; for amplification of microsatellites, these samples are diluted down to 40 ng/µL.

Further Procedures and Analysis

To continue this study, I will amplify Msd029, Msd036, Msd072, and Msd029 microsatellite markers (*Xiphophorus* Genetic Stock Center) using PCR. These markers were chosen because previously they showed a high degree of polymorphism in two populations of *X.birchmanni* (Paczolt et al. *in Review*). I will determine the genotypes of the offspring and parents using the program, Peakscanner (Life Technologies). Then I will use the computer program GEURD (Genetic Evaluation and Reconstruction of parental genotypes using multilocus DNA data, Jones 2005) to determine the number of potential sires for each brood.

Expected Results

I expect that this population will display a high level of multiple paternity. According to Table 1, several species of *Xiphophorus* display high levels of multiple paternity, including *X. birchmanni*. This table allows us to have an idea about the levels we should expect to see in our hybrid population.

Table 1: Represents a list describing multiple paternity in representative *Xiphophorus* species. The average number of sires and percent of broods with multiple paternity within study samples are present, including results from *X. birchmanni*, which is a parental species in this hybrid system.

Species	$N_{families}$	% multiply sired	$Mean_{sires}$	Reference
X. maculatus	71 (2 pops)	66	1.9	Borowsky & Kallman 1976
X. variatus	43	42	1.4	Borowsky & Khouri 1976
X. multilineatus	18	33	1.4	Luo et al. 2005
X. hellerii	14	71	1.6-2.3*	Simmons et al. 2008
X. hellerii	69 (2 pops)	64	1.8	Tatarenkov et al. 2008
X. birchmanni	43 (3 pops)	84	2.37	Paczolt et al. (in prep)

Further Implications

The purpose of this study was to determine multiple paternity analysis in a natural hybrid zone between two *Xiphophorus* species. Research in numerous other systems has found that when females mate multiply, it introduces additional considerations to how sexual selection acts on males. Polyandry can increase competition in males due to the increasing difficulty of having a successful mating encounter with females, and can thus potentially decrease precopulatory sexual selection why increasing the intensity of postcopulatory sexual selection. Especially considering that females of the genus *Xiphophorus* can also store sperm for many months after mating, so sexual selection that occurs after mating in this fish could be quite fierce. This study, while it does not reveal how many times a female actually mates, will tell us at least how many males are usually successful in mating attempts with females, which is a key piece of

information for eventually determining how postcopulatory sexual selection might act in this hybrid zone. Much work has been done to understand pre copulatory preferences and mate choice, so this study will allow for new discoveries and a greater understanding of sexual selection as a whole in these species and their hybrids.

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